

ORGANICS LABORATORY

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Method No. D25011	Edition 08/12/91	Revision 5
Subject: Determination of Dicamba and Dichlorosalicylic Acid (DCSA) in Soil		
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Approved By: Kim Sjogren		
References: SCPC, Method #AM-0766A		

1.0 SCOPE

This method describes the procedure for extraction, cleanup, and quantitation of Dicamba and DCSA in soil with a detection limit of 0.01 ppm.

The modifications to the original Sandoz method include: only butyl esters are derivitized, the indicator tube from the esterification step is not usually used, 1.5 ml of carbitol and 1.5 ml ethyl ether is used instead of 1.4 ml during the esterification step, and actual GC conditions, such as temperature, vary from the original method.

2.0 PRINCIPLE

This method permits the simultaneous determination of Dicamba and DCSA as individual residues or combined as Dicamba residue. The Dicamba and DCSA are extracted from the soil sample by treatment with aqueous alkali solution at 65°C followed by 15 minutes of shaking. The mixture is centrifuged and an aliquot of the extract is acidified to a pH less than 1. The residues are partitioned into ethyl ether, derivitized, cleaned up and quantitated by GC/EC. The limit of detection for each compound is 0.01 ppm.

3.0 CHEMICALS AND SOLUTIONS

Butanol - reagent, Sigma, St. Louis, MO

1-Butyl-3-nitro-1-nitrosoguanidine: Aldrich Chemical Co.,
Milwaukee, WI 53233

Carbitol, 2-(2-ethoxyethoxy) ethanol: Aldrich Chemical Co.

Diazald, Aldrich Chemical Co.

Ethyl Ether: Mallinckrodt (HPLC grade), Paris, KY

Hexane: reagent, J. T. Baker, Phillipsburg, PA

Methanol: Baker Resi-analyzed, J. T. Baker Chemical Co.

Pentane: "distilled in glass," Burdick and Jackson,
Muskegon, IL

Potassium Chloride, reagent grade.

Potassium Hydroxide, reagent grade.

Silica gel 60, 70-200 mesh: EM reagent, MC/B Manufacturing
Chemists Inc., Cincinnati, OH

Sodium Chloride, reagent grade.

Sodium Sulfate, anhydrous granular, reagent grade.

Sulfuric Acid, reagent grade.

Extracting Solution: 0.5 N KOH/10% KCl solution (28.1 g KOH
+ 100.0 g KCl in 1000 ml DI H₂O)

6 N H₂SO₄: 16.7 ml H₂SO₄ to 100 ml volume with DI H₂O

9 N KOH Solution: 50.49 g KOH in 100 ml DI H₂O

5% Ethyl Ether in Pentane Solution: 50 ml Ethyl Ether + 950
ml Pentane.

Silica Gel Preparation: Activate 500+ g silica gel at 250+
°C for 0.5+ hours. Transfer to 2000 ml plastic bottle, cap
with aluminum foil and lid, and shake or turbulate for
approximately 0.5 hr. Weigh silica gel and deactivate with
DI H₂O (485 g silica gel + 15 g DI H₂O). Shake or turbulate
continuously for 6 - 15 hours. This silica gel preparation
is good for approximately 2 weeks.

4.0 EQUIPMENT

Bath, water: approx. 65 °C

Bottle, glass centrifuge: poly-seal screw cap

Centrifuge: International, Scientific Supply, Chicago, IL

Chromatograph, Gas: Shimadzu GC-14A and Automatic Sample
Injector AOC-9, with Electron Capture Detector, or
equivalent.

Column: Methyl Silicon Fused Silica Capillary Column,
Quadrex Corporation, Catalog #007-1-25W-0.5F, or
equivalent
Length: 25 m
I. D.: 0.32 mm
Film Thickness: 0.5 μ m

Temperature: 175 °C

Columns, Chromatographic: Kimax 15 mm (id) x 45 cm teflon
stopcock, VWR Scientific, or equivalent.

Concentrator, Kuderna-Danish: 125, 250 or 500-ml K-D flask
with 10-14 ml graduated distillation receiver tube and
Vigreux column (24/40, 38.1 cm) or equivalent.

Cylinders, graduated: 10-ml, 100-ml and 1000-ml.

Esterification apparatus: see diagram page 6.

Flask, volumetric: 5-ml, 50-ml, 100-ml.

Funnel, filter: 60 degree or equivalent

Funnel, separatory: 125-ml with teflon stopcock.

Glass wool, silanized.

Nitrogen with bubbler apparatus

Oven: 250+ °C

Paper, pH: range 1-14

Pipets, volumetric: 1-ml, 5-ml, 20-ml

Shaker, wrist-action and/or turbulating apparatus

Tube, test: 18 X 145 mm or equivalent.

5.0 ANALYTICAL PROCEDURE

5.1 Extraction and Partition

- 5.1.1 Weigh on a calibrated scale 20.000 +/- 0.020 g of soil into a glass centrifuge screw-cap bottle. Fortify spikes at this point with 1 ml of an appropriate spiking solution, and let dry (10 - 20 min.). Add 80 ml of 0.5 N KOH/10% KCl solution.

- 5.1.2 Place bottle in a 60-95 °C water bath for approximately 15 minutes with cap removed. The bottle may be covered with a square of aluminum foil.
- 5.1.3 Remove bottle from water bath and cool to room temperature (cold water bath may be used).
- 5.1.4 Cap bottle tightly with both aluminum foil and cap and shake for approximately 15 minutes on a mechanical wrist-action shaker.
- 5.1.5 Centrifuge for approximately 10 minutes at 1200-1500 RPM.
- 5.1.6 Transfer a 20 ml aliquot of extract (pipet) to another glass centrifuge bottle and add 5 ml 6N sulfuric acid, approximately 15 g^l sodium chloride and 100 ml ethyl ether. The pH of the aqueous phase should be <1. (If not, add more 6N sulfuric acid). CAUTION: solution must be cooled to room temperature before addition of ether.
- 5.1.7 Cap (+ aluminum foil) the bottle and shake the mixture for approximately 15 minutes on mechanical wrist-action shaker.
- 5.1.8 Centrifuge (see 5.1.5) to break the emulsion and transfer contents to a 125 ml separatory funnel.
- 5.1.9 Discard aqueous (lower) layer and pass ether through anhydrous sodium sulfate into a Kuderna-Danish concentrator (see 4.1 Equipment). Rinse funnel and sodium sulfate with 15 to 20 ml ether.
- 5.1.10 Concentrate the ether to approximately 1-7 ml in the water bath (approximately 65 °C).

5.2 Derivatization

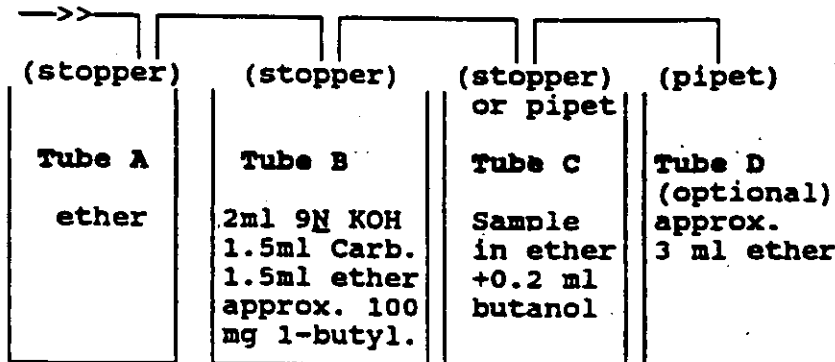
5.2.1 Butylation

- 5.2.1.1 Set up tubes as in the diagram on page 6. Tube A serves to saturate the nitrogen flow with ether which transmits diazobutane from B to C. Tube D is used as an indicator for excess diazobutane when the sample extract is colored. (Tube D is optional).

- 5.2.1.2 To Tube B (18 X 145 mm test tube or equivalent), pipet 2 ml 9 N KOH, 1.5 ml Carbitol, and 1.5 ml ether.
- 5.2.1.3 To concentrator Tube C which contains the sample extract, add 0.2 ml butanol.
- 5.2.1.4 To Tube D (optional) add approximately 3 ml ether.
- 5.2.1.5 Adjust nitrogen flow rate to insure a steady, light flow bubbling through the tubes. Add approximately 100 mg¹ 1-butyl-3-nitro-1-nitrosoguanidine to Tube B and seat stoppers in Tubes A, B, and C. Note: If Tube D is not being used, use a disposable pipet to deliver the diazobutane from the hose that leaves Tube B and connects Tube C, the sample tube). When yellow color is obtained in Tube C, stop nitrogen flow and rinse pipet with a few drops of ether into the sample container (Tube C). For extremely colored extracts, Tube D may be needed to determine yellow color (butylation). NOTE: 1-Butyl-3-nitro-1-nitrosoguanidine is a carcinogen. In addition, the diazo-butane gas generated is extremely toxic and should only be used in well-ventilated hoods. Do not use ground glass connections when working with diazobutane.
- 5.2.1.6 Cap the condenser tube for a few minutes (teflon stopcock or alum. foil). If solution remains yellow throughout this interval, evaporate the sample to near dryness under a gentle stream of nitrogen in a well-ventilated hood, using no heat. Proceed to 5.3.
- 5.2.1.7 If the yellow color disappears quickly, recharge the extract with diazobutane.

ESTERIFICATION APPARATUS:

Nitrogen Flow



5.2.2 Methylation

5.2.2.1 Same as described for butylation except use methanol and Diazald in place of butanol and 1-butyl-3-nitro-1-nitrosoquinidine.

5.3 Column Cleanup

5.3.1 Butyl Esters

5.3.1.1 Set up the chromatography columns. Fit each with a glass wool plug to contain the silica gel, and close the stopcock.

5.3.1.2 To a 250-ml separatory funnel add 70 ml of 5% ethyl ether in pentane (5% eluent) and approximately 20 g¹ of 3% water deactivated silica gel. (See Chemicals and Solutions.)

5.3.1.3 Shake well (VENT!) and quickly transfer slurry to the column.

5.3.1.4 Rinse the separatory funnel with 10 ml 5% eluent and add to the column.

5.3.1.5 Cover the silica gel bed with approximately 1 cm granular anhydrous sodium sulfate and drain the solvent just to the sodium sulfate level.

5.3.1.6 Add 5 ml of the 5% eluent to the butylated sample and transfer to the silica gel column.

- 5.3.1.7 Rinse the condenser tube with two 5-ml portions of 5% eluent, and allow each rinse to penetrate to the top of the bed.
- 5.3.1.8 Pass another portion of 5% eluent through the column, for a total of 20 ml, and discard. Collect the butyl esters of dicamba and DCSA with 110 ml 5% eluent, into a 125-ml boiling flask.
- 5.3.1.9 Transfer the sample to a K-D apparatus and boil down, using a steam bath or equivalent, to less than five ml. Quantitatively transfer to a 2 or 5 ml volumetric flask, rinse K-D with small proportions of 5% eluent, and bring to volume. Optional: A gentle stream of nitrogen and a warm water bath (approx. 45 °C) may be used to finish the final reduction of sample volume to avoid the sample going to dryness.

6.0 ANALYSIS

6.1 Gas Chromatographic Conditions

- 6.1.1 Instrument: Shimadzu GC-14A with Electron Capture Detector, or equivalent.
- 6.1.2 Autosampler: Shimadzu AOC-9 serial #81450 YS, or equivalent.
- 6.1.3 Column: Methyl Silicon Fused Silica Capillary Column, Quadrex Corporation, Catalog # 007-1-25W-0.5F, or equivalent
Length: 25 m
I.D.: 0.32 mm
Film Thickness: 0.5 µm
- 6.1.4 Detector: Ni-63 Electron Capture
Temperature: 275 °C
Make up Gas: Nitrogen, Ultra High Purity Grade
Flow: 40 mL/min.
- 6.1.5 Inlet Temp: 235-240 °C
- 6.1.6 Split Ratio: 6:1 or 5:1
- 6.1.7 Carrier Gas: Hydrogen, Ultra High Purity Grade

6.1.8 Temperature Program:

Initial Temperature: 150-175 °C
Initial Time: 8-10 minutes
Ramp: 20-30 °C/minute
Final Temp: 250 °C
Hold Time: 5 min.; Cool Down

The temperature program should be varied between these parameters to allow for the separation of possible organic interferences.

6.1.9 Injection Volume: 4 or 5 µL

6.1.10 Approximate Retention Times:

Dicamba Butylester: 3.2 min. (170°C),
6.4 min. (150°C, ramp 20°/min. @ 8 minutes)

DCSA Butylester: 7.1 min. (170°C),
10.7 min. (150°C, ramp 20°/min. @ 8 minutes)

6.1.11 Current: 1 nA

6.1.12 Data System: Waters 860 system, or equivalent.

6.2 Standards

6.2.1 Dicamba reference standard #RS-M36-030388 of 99.8% purity obtained from Sandoz Crop Protection, 1300 E. Touhy Ave, Des Plaines, IL.

6.2.2 3,6-dichlorosalicylic acid (DCSA) reference standard #RS-36DCSA-101890 of 99.5% purity obtained from Sandoz Crop Protection, 1300 E. Touhy Ave, Des Plaines, IL.

6.2.3 Dicamba and DCSA powder should be stored frozen. Stock and working solutions should be stored refrigerated.

6.3 Determination of Dicamba and DCSA

6.3.1 Preparation of Standard Solutions (Butyl Esters)

6.3.1.1 Weigh on a calibrated scale 100.0 mg ± 0.8 of dicamba acid and 100.0 mg ± 0.8 of DCSA, and transfer to a 100-ml volumetric flask with methanol, diluting to volume. The concentration of the stock solution is approximately 1000 µg/ml.

- 6.3.1.2 Pipet 1 ml of stock solution to a 100 ml volumetric flask and dilute with methanol to the mark. The concentration of this solution is approximately 10 $\mu\text{g/ml}$.
 - 6.3.1.3 Butylate 2 ml of the 10 PPM solution as described in 5.2.1. Concentrate under a gentle stream of nitrogen in a well-ventilated hood to approximately 1 ml.
 - 6.3.1.4 Transfer the butylated compound to a 100 ml volumetric flask and adjust volume to the mark with pentane. The concentration of GC standard is approximately 0.20 $\mu\text{g/ml}$. Serial dilutions are then made from this 0.20 PPM standard as desired.
- 6.3.2 Preparation of Standard Solutions (Methyl Esters)
- 6.3.2.1 Same as described in 6.3.1, except methylate as described in 5.2.2.
- 6.4 Quantitation
- 6.4.1 Inject 4-5 μL of known amount of Dicamba/DCSA standards onto the GC.
 - 6.4.2 Measure the peak response.
 - 6.4.3 Plotting peak response versus concentration, calculate the best fit line using quadratic through zero regression.
 - 6.4.4 Inject 4-5 μL of sample extract onto the GC. Measure peak response.

6.4.5 Determine the concentration ($\mu\text{g/g}$) of Dicamba and DCSA in the sample by the equation:

$$\mu\text{g/ml} = \frac{\text{ng} \times V_s}{\mu\text{L} \times W}$$

where:

- ng = quantity of compound in injected aliquot as read from the standard curve in nanograms.
- V_s = volume of sample extract in milliliters from which GC aliquot was taken, allowing for all dilutions.
- μL = volume of sample extract injected onto gas chromatograph in microliters.
- W = weight of the sample taken for analysis in grams.

¹ Amounts may be weighed the first time. The remaining samples can be measured by visual comparison.

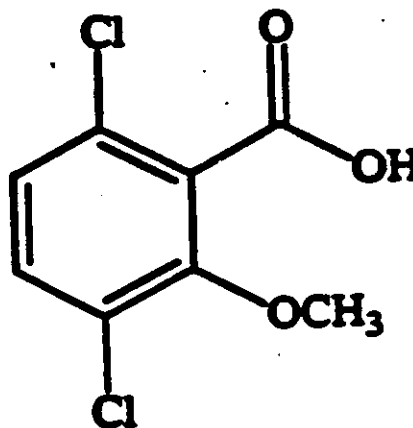
Dicamba

TABLE 1 - PHYSICAL/CHEMICAL PROPERTIES

A. IDENTITY

Empirical Formula: $C_8H_6Cl_2O_3$

Structural Formula:



B. PHYSICAL/CHEMICAL PROPERTIES

Molecular Weight:	221.04
Physical State:	crystalline solid
Color:	light tan
Odor:	slightly phenolic
Melting Point:	90 - 100°C
Boiling Point:	decomposes
Density/Specific Gravity @ 25/25°C:	1.57
Vapor Pressure:	3.41×10^{-5}
Dissociation Constant:	1.16×10^{-2}
Octanol/Water Coefficient:	3.5
pH:	2.5 - 3.0
Flash Point:	150°C
Corrosion Characteristics:	slight
Solubility (25°C) grams/100 ml:	

<u>Solvent</u>	<u>Solubility</u>
Water	0.61
Ethanol	92.2
Hexane	5.2
Xylene	7.8
Isopropyl Alcohol	76.0
Acetone	81.0
Toluene	13.0
Methylene Chloride	26.0
Dioxane	118.0

K_{oc} : .511

Soil Half-life: < 25 days

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